```
FILE 'REGISTRY' ENTERED AT 15:07:44 ON 08 MAY 2002

=> S CITRATE LYASE/CN
L1             1 CITRATE LYASE/CN

=> D

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2002 ACS
RN   9012-83-3  REGISTRY
CN   Lyase, citrate (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Citrase
CN   Citratase
CN   Citrate aldolase
CN     ***Citrate lyase***
CN   Citric aldolase
CN   Citridesmolase
CN   Citritase
CN   CL 400
CN   E.C. 4.1.3.6
MF   Unspecified
CI   MAN
LC   STN Files:   AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS,
       CHEMCATS, CHEMLIST, EMBASE, PROMT, TOXCENTER, USPATFULL
     Other Sources:   EINECS**
         (**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
             225 REFERENCES IN FILE CA (1967 TO DATE)
               2 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
             225 REFERENCES IN FILE CAPLUS (1967 TO DATE)


FILE 'CAPLUS' ENTERED AT 15:08:11 ON 08 MAY 2002

=> S CITRATE LYASE;S (CIRTRATE OR CITRIC)(W)ALDOLASE;S CITRATASE;S CITRASE;S CITRIDESMOLASE;S
CITRITASE;S L1
          69686 CITRATE
           1984 CITRATES
          70649 CITRATE
                  (CITRATE OR CITRATES)
          12894 LYASE
           1639 LYASES
          13337 LYASE
                  (LYASE OR LYASES)
L2         1090 CITRATE LYASE
                  (CITRATE(W)LYASE)


             1 CIRTRATE
          62489 CITRIC
             2 CITRICS
          62491 CITRIC
                  (CITRIC OR CITRICS)
           9395 ALDOLASE
           1636 ALDOLASES
           9568 ALDOLASE
                  (ALDOLASE OR ALDOLASES)
L3            0 (CIRTRATE OR CITRIC)(W)ALDOLASE


L4            6 CITRATASE


L5           10 CITRASE


L6            3 CITRIDESMOLASE


L7           21 CITRITASE
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L8          225 L1

=> S L2,L4,L5,L6,L7,L8
L9         1158 (L2 OR L4 OR L5 OR L6 OR L7 OR L8)

=> S CITC OR CIT(W)C;S CITD OR CIT(W)D;S CITE OR CIT(W)E;S CITF OR CIT(W)F;S CITG OR CIT(W)G;S
CITX OR CIT(W)X
            27 CITC
         14991 CIT
           101 CITS
         15087 CIT
                 (CIT OR CITS)
       2891675 C
            36 CIT(W)C
L10         63 CITC OR CIT(W)C


             9 CITD
         14991 CIT
           101 CITS
         15087 CIT
                 (CIT OR CITS)
       1846343 D
            15 CIT(W)D
L11         24 CITD OR CIT(W)D


           424 CITE
           774 CITES
          1197 CITE
                 (CITE OR CITES)
         14991 CIT
           101 CITS
         15087 CIT
                 (CIT OR CITS)
       1603560 E
             8 CIT(W)E
L12       1205 CITE OR CIT(W)E


            17 CITF
             1 CITFS
            17 CITF
                 (CITF OR CITFS)
         14991 CIT
           101 CITS
         15087 CIT
                 (CIT OR CITS)
        508474 F
             1 CIT(W)F
L13         18 CITF OR CIT(W)F


           26 CITG
         14991 CIT
           101 CITS
         15087 CIT
                 (CIT OR CITS)
       2476375 G
             2 CIT(W)G
L14         28 CITG OR CIT(W)G


            3 CITX
         14991 CIT
           101 CITS
         15087 CIT
                 (CIT OR CITS)
       1235136 X
             6 CIT(W)X
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L15          9 CITX OR CIT(W)X

=> S L10 AND L11 AND L12 AND L13 AND L14 AND L15
L16          0 L10 AND L11 AND L12 AND L13 AND L14 AND L15

=> S (ESCHERICHIA OR E)(W)COLI
        202081 ESCHERICHIA
             1 ESCHERICHIAS
        202082 ESCHERICHIA
                 (ESCHERICHIA OR ESCHERICHIAS)
       1603560 E
        218290 COLI
            11 COLIS
        218298 COLI
                 (COLI OR COLIS)
L17     211334 (ESCHERICHIA OR E)(W)COLI

=> S L17 AND L9
L18         47 L17 AND L9

=> S L18 AND L15
L19          3 L18 AND L15

=> D 1-3 CBIB ABS

L19  ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2002 ACS
2000:736049   Document No. 134:97157  Identification of triphosphoribosyl-
     dephospho-CoA as precursor of the   ***citrate***     ***lyase***
     prosthetic group.  Schneider, K.; Dimroth, P.; Bott, M. (Institut fur
     Mikrobiologie, Eidgenossische Technische Hochschule Zurich, Zurich,
     CH-8092, Switz.).  FEBS Letters, 483(2,3), 165-168 (English) 2000.  CODEN:
     FEBLAL.  ISSN: 0014-5793.  Publisher: Elsevier Science B.V..
AB   The .gamma.-subunit of   ***citrate***     ***lyase***   (EC 4.1.3.6)
     contains the prosthetic group 2'-(5''-phosphoribosyl)-3'-dephospho-CoA and
     serves as an acyl carrier protein (ACP).  We recently showed that in
       ***Escherichia***     ***coli***   the proteins CitG and   ***CitX***
     are essential for holo-ACP synthesis and provided evidence that CitG
     catalyzes the formation of a prosthetic group precursor from ATP and
     dephospho-CoA, which is subsequently attached via phosphodiester linkage
     to apo-ACP by   ***CitX***   .  Here we prove that CitG indeed catalyzes
     the conversion of ATP and dephospho-CoA to adenine and
     2'-(5''-triphosphoribosyl)-3'-dephospho-CoA, the predicted precursor of
     the prosthetic group.  Furthermore, this precursor was transferred by
       ***CitX***   to apo-ACP, yielding holo-ACP.  Thus, our proposed mechanism
     for holo-ACP synthesis could be verified.

L19  ANSWER 2 OF 3  CAPLUS  COPYRIGHT 2002 ACS
2000:682471   Document No. 134:26900  Identification of the Active Site of
     Phosphoribosyl-dephospho-coenzyme A Transferase and Relationship of the
     Enzyme to an Ancient Class of Nucleotidyltransferases.  Hoenke, Stefan;
     Schmid, Markus; Dimroth, Peter (Institut fuer Mikrobiologie,
     Eidgenoessischen Technischen Hochschule Zuerich, Zurich, CH-8092, Switz.).
     Biochemistry, 39(43), 13233-13240 (English) 2000.  CODEN: BICHAW.  ISSN:
     0006-2960.  Publisher: American Chemical Society.
AB   Malonate decarboxylase from Klebsiella pneumoniae contains an acyl carrier
     protein (MdcC) to which a 2'-(5''-phosphoribosyl)-3'-dephospho-CoA
     prosthetic group is attached via phosphodiester linkage to serine 25.  We
     have shown in the preceding paper in this issue that the formation of this
     phosphodiester bond is catalyzed by a phosphoribosyl-dephospho-CoA
     transferase MdcG with the substrate 2'-(5''-triphosphoribosyl)-3'-
     dephospho-CoA that is synthesized from ATP and dephospho-CoA by the
     triphosphoribosyl transferase MdcB.  The reaction catalyzed by MdcG is
     related to nucleotidyltransfer reactions, and the enzyme indeed catalyzes
     unphysiol. nucleotidyltransfer, e.g., adenylyltransfer from ATP to apo
     acyl carrier protein (ACP).  These unspecific side reactions are favored
     at high Mg2+ concns.  A sequence motif including D134 and D136 of MdcG is
     a signature of all nucleotidyltransferases.  It is known from the
     well-characterized mammalian DNA polymerase that this motif is at the
     active site of the enzyme.  Site-directed mutagenesis of D134 and/or D136
     of MdcG to alanine abolished the transfer of the prosthetic group to apo
     ACP, but the binding of triphosphoribosyl-dephospho-CoA to MdcG was not
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affected. Evidence is presented that similar to MdcG, MadK encoded by the malonate decarboxylase operon of Malonomonas rubra and ***CitX*** from the operon encoding ***citrate*** ***lyase*** in ***Escherichia*** ***coli*** are phosphoribosyl-dephospho-CoA transferases catalyzing the attachment of the phosphoribosyl-dephospho-CoA prosthetic group to their specific apo ACPs.

L19 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2002 ACS
2000:461360 Document No. 133:234441 Biosynthesis of the Prosthetic Group of ***Citrate*** ***Lyase*** . Schneider, Karin; Dimroth, Peter; Bott, Michael (Institut fuer Mikrobiologie, Eidgenoessische Technische Hochschule Zuerich, Zurich, CH-8092, Switz.). Biochemistry, 39(31), 9438-9450 (English) 2000. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB ***Citrate*** ***lyase*** (EC 4.1.3.6) catalyzes the cleavage of citrate to acetate and oxaloacetate and is composed of three subunits (.alpha., .beta., and .gamma.). The .gamma.-subunit serves as an acyl carrier protein (ACP) and contains the prosthetic group 2'-(5''-phosphoribosyl)-3'-dephospho-CoA, which is attached via a phosphodiester linkage to serine-14 in the enzyme from Klebsiella pneumoniae. In this work, we demonstrate by genetic and biochem. studies with ***citrate*** ***lyase*** of ***Escherichia*** ***coli*** and K. pneumoniae that the conversion of apo-ACP into holo-ACP is dependent on the two proteins, ***CitX*** (20 kDa) and CitG (33 kDa). In the absence of ***CitX*** , only apo-ACP was synthesized in vivo, whereas in the absence of CitG, an adenylylated ACP was produced, with the AMP residue attached to serine-14. The adenylyltransferase activity of ***CitX*** could be verified in vitro with purified ***CitX*** and apo-ACP plus ATP as substrates. Besides ATP, CTP, GTP, and UTP also served as nucleotidyl donors in vitro, showing that ***CitX*** functions as a nucleotidyltransferase. The conversion of apo-ACP into holo-ACP was achieved in vitro by incubation of apo-ACP with ***CitX*** , CitG, ATP, and dephospho-CoA. ATP could not be substituted with GTP, CTP, UTP, ADP, or AMP. In the absence of CitG or dephospho-CoA, AMP-ACP was formed. Remarkably, it was not possible to further convert AMP-ACP to holo-ACP by subsequent incubation with CitG and dephospho-CoA. This demonstrates that AMP-ACP is not an intermediate during the conversion of apo- into holo-ACP, but results from a side activity of ***CitX*** that becomes effective in the absence of its natural substrate. Our results indicate that holo-ACP formation proceeds as follows. First, a prosthetic group precursor [presumably 2'-(5''-triphosphoribosyl)-3'-dephospho-CoA] is formed from ATP and dephospho-CoA in a reaction catalyzed by CitG. Second, holo-ACP is formed from apo-ACP and the prosthetic group precursor in a reaction catalyzed by ***CitX*** .

=> S KLEBSIELLA PNEUMONIAE;S LEUCONOSTOC MESENTEROIDES;S HAEMOPHILUS INFLUENZAE
```
        13067 KLEBSIELLA
           10 KLEBSIELLAS
        13067 KLEBSIELLA
                (KLEBSIELLA OR KLEBSIELLAS)
        17712 PNEUMONIAE
L20      8404 KLEBSIELLA PNEUMONIAE
                (KLEBSIELLA(W)PNEUMONIAE)


         3654 LEUCONOSTOC
           72 LEUCONOSTOCS
         3673 LEUCONOSTOC
                (LEUCONOSTOC OR LEUCONOSTOCS)
         2229 MESENTEROIDES
L21      2105 LEUCONOSTOC MESENTEROIDES
                (LEUCONOSTOC(W)MESENTEROIDES)


         7839 HAEMOPHILUS
         6294 INFLUENZAE
L22      5809 HAEMOPHILUS INFLUENZAE
                (HAEMOPHILUS(W)INFLUENZAE)
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=> S L20 AND L9;S L21 AND L9;S L22 AND L9
L23 26 L20 AND L9

L24 12 L21 AND L9

L25 9 L22 AND L9

=> S (L23,L24,L25) NOT L19
L26 37 ((L23 OR L24 OR L25)) NOT L19

=> D 1-37 CBIB ABS

L26 ANSWER 1 OF 37 CAPLUS COPYRIGHT 2002 ACS
2002:89878 Document No. 136:156403 Methods for identifying therapeutic targets for treating infectious disease. Shepard, Michael H.; Lackey, David B.; Cathers, Brian E.; Sergeeva, Maria V. (Newbiotics, Inc., USA). PCT Int. Appl. WO 2002007780 A2 20020131, 503 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US23095 20010720. PRIORITY: US 2000-PV219598 20000720; US 2000-PV244953 20001101; US 2001-PV276728 20010316.
AB This invention provides methods and systems to identify enzymes that act as enzyme-catalyzed therapeutic activators and the enzymes identified by these methods. Also provided by this invention are compds. activated by the enzymes as well as compns. contg. these compds.

L26 ANSWER 2 OF 37 CAPLUS COPYRIGHT 2002 ACS
2001:758328 Document No. 136:196116 Characterization of sensor kinase CitA from ***Klebsiella*** ***pneumoniae*** and Escherichia coli as a citrate receptor. Kaspar, Sibylle (Inst. Biotechnologie, Germany). Berichte des Forschungszentrums Juelich, Juel-3851, i-xi, 1-119 (German) 2001. CODEN: FJBEE5. ISSN: 0366-0885.
AB Sensor-kinases CitA were characterized of the two-component regulatory systems CitA/CitB of ***Klebsiella*** ***pneumoniae*** and E. coli, which regulate expression of citrate fermn. genes. C-terminal kinase domain and periplasmic domain of sensor-kinases CitA of both enterobacteria were investigated. Isolated kinase domain of K. pneumoniae showed constitutive autokinase activity and transmitted the .gamma.-phosphate group of ATP to the 'response'-regulator CitB. His-350 and aspartate-56 were identified as probable positions of phosphorylation of CitA resp. CitB. Citrate binding was detected with the periplasmic domain of K. pneumoniae, but not with other tested tri- or dicarboxylates. Cit A of K. pneumoniae was discussed to be the prototype of a family of sensor-kinases, whose amino acid sequences are conserved in the C-terminal kinase domain, periplasmic domain, and 'linker'-domain. Two other members of the family, sensor-kinases CitA and DcuS of E. coli, were characterized. Binding studies using isothermal titrn. calorimetry studies showed that the periplasmic domain of sensor-kinase CitA of E. coli acted as a citrate receptor. Expression of genes of fumarate respiration was discussed to be regulated by the DcuS/DcuR-system (sensor-kinase and 'response'-regulator of E. coli), but binding of fumarate or succinate to the periplasmic domain of sensor-kinase DcuS was not detectable unambiguously.

L26 ANSWER 3 OF 37 CAPLUS COPYRIGHT 2002 ACS
2001:658950 Document No. 136:242764 Catabolite repression of the citrate fermentation genes in ***Klebsiella*** ***pneumoniae*** : evidence for involvement of the cyclic AMP receptor protein. Meyer, Margareta; Dimroth, Peter; Bott, Michael (Institut fur Mikrobiologie, Eidgenossische Technische Hochschule Zurich, Zurich, 8092, Switz.). Journal of Bacteriology, 183(18), 5248-5256 (English) 2001. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.
AB ***Klebsiella*** ***pneumoniae*** is able to grow anaerobically

with citrate as a sole carbon and energy source by a fermentative pathway involving the Na+-dependent citrate carrier CitS, ***citrate*** ***lyase*** , and oxaloacetate decarboxylase. The corresponding genes are organized in the divergent citC and citS operons, whose expression is strictly dependent on the citrate-sensing CitA-CitB two-component system. Evidence is provided here that the citrate fermn. genes are subject to catabolite repression, since anaerobic cultivation with a mixt. of citrate and glucose or citrate and gluconate resulted in diauxic growth. Glucose, gluconate, and also glycerol decreased the expression of a chromosomal citS-lacZ fusion by 60 to 75%, whereas a direct inhibition of the citrate fermn. enzymes was not obsd. The purified cAMP (cAMP) receptor protein (CRP) of K. pneumoniae bound to two sites in the citC-citS intergenic region, which were centered at position -41.5 upstream of the citC and citS transcriptional start sites. Binding was apparently stimulated by the response regulator CitB. These data indicate that catabolite repression of the citrate fermn. genes is exerted by CRP and that in the absence of repressing carbon sources the cAMP-CRP complex serves to enhance the basal, CitB-dependent transcription level.

L26 ANSWER 4 OF 37 CAPLUS COPYRIGHT 2002 ACS
2001:578309 Document No. 136:101411 Plasmid profile and characterization on negative mutants for lactose and citrate metabolism derived from ***Leuconostoc*** ***mesenteroides*** . Sewaki, Tomomitsu; Tagawa, Yuji; Miyamoto, Taku (Fac. Agric., Okayama Univ., Okayama-shi, 700-8530, Japan). Miruku Saiensu, 50(2), 49-54 (Japanese) 2001. CODEN: MISAFD. ISSN: 1343-0289. Publisher: Nippon Rakuno Kagakkai.
AB Lactose- and citrate-neg. (Lac- and Cit-) mutants were isolated after the treatment of ***Leuconostoc*** ***mesenteroides*** strains 6-1-9 and OR-2 with acridine orange and examd. for their plasmid profiles and enzymic characteristics. Lac- mutants, designated 6-1-9-1 and 6-1-9-2 were deficient a 38 Mdal plasmid and they lost activities of the lactose-splitting enzyme (.beta.-galactosidase). On the other hand Cit- mutant (OR-2-1) missing a 15 Mdal plasmid lost the citrate permease activity, although it possessed less ***citrase*** activity.

L26 ANSWER 5 OF 37 CAPLUS COPYRIGHT 2002 ACS
2001:261134 Document No. 134:276507 Production of ***citrate*** ***lyase*** with recombinant microorganisms expressing the citCDEF(X)G gene cluster. Dimroth, Peter; Bott, Michael; Schneider, Karin (Roche Diagnostics G.m.b.H., Germany; ETH Zurich). Eur. Pat. Appl. EP 1090988 A1 20010411, 22 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (German). CODEN: EPXXDW. APPLICATION: EP 1999-119404 19990930.
AB Enzymically active ***citrate*** ***lyase*** is produced with microorganisms expressing the citCDEFG operon of ***Haemophilus*** ***influenzae*** , ***Klebsiella*** ***pneumoniae*** , or ***Leuconostoc*** ***mesenteroides*** or the citCDEFXG operon of Escherichia coli. The recombinant enzyme may be used, in the presence of NAD and L-malate dehydrogenase or L-lactate dehydrogenase, for detn. of citric acid. Thus, cell-free lysates of recombinant E. coli contg. 4-5 u/mg protein were produced.

L26 ANSWER 6 OF 37 CAPLUS COPYRIGHT 2002 ACS
2001:246610 Document No. 134:276495 Production of ***citrate*** ***lyase*** with recombinant microorganisms expressing the citCDEF(X)G gene cluster. Bott, Michael; Dimroth, Peter; Schneider, Karin (Roche Diagnostics G.m.b.H., Germany). Eur. Pat. Appl. EP 1088886 A1 20010404, 22 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (German). CODEN: EPXXDW. APPLICATION: EP 2000-120988 20000927. PRIORITY: EP 1999-119404 19990930.
AB Enzymically active ***citrate*** ***lyase*** is produced with microorganisms expressing the citCDEFG operon of ***Haemophilus*** ***influenzae*** , ***Klebsiella*** ***pneumoniae*** , or ***Leuconostoc*** ***mesenteroides*** or the citCDEFXG operon of Escherichia coli. The recombinant enzyme may be used, in the presence of NAD and L-malate dehydrogenase or L-lactate dehydrogenase, for detn. of citric acid. Thus, cell-free lysates of recombinant E. coli contg. 4-5 u/mg protein were produced.

L26 ANSWER 7 OF 37 CAPLUS COPYRIGHT 2002 ACS
1999:453811 Document No. 131:209867 Genetic organization of the citCDEF

locus and identification of mae and clyR genes from ***Leuconostoc*** ***mesenteroides*** . Ali, Sadjia Bekal-Si; Divies, Charles; Prevost, Herve (Laboratoire de Microbiologie, UA INRA, Universite de Bourgogne ENS.BANA, Dijon, F-21 000, Fr.). Journal of Bacteriology, 181(14), 4411-4416 (English) 1999. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB In this paper, the authors describe two open reading frames coding for a NAD-dependent malic enzyme (mae) and a putative regulatory protein (clyR) found in the upstream region of citCDEFG of ***Leuconostoc*** ***mesenteroides*** subsp. cremoris 195. The transcriptional anal. of the ***citrate*** ***lyase*** locus revealed one polycistronic mRNA covering the mae and citCDEF genes. This transcript was detected only on RNA prepd. from cells grown in the presence of citrate. Primer extension expts. suggest that clyR and the ***citrate*** ***lyase*** operon are expressed from a bidirectional A-T-rich promoter region located between mae and clyR.

L26 ANSWER 8 OF 37 CAPLUS COPYRIGHT 2002 ACS

1998:635867 Document No. 130:206 Strategies towards a better understanding of antibiotic action. Folate pathway inhibition in Hemophilus influenzae as an example. Evers, Stefan; Di Padova, Karin; Meyer, Michelle; Fountoulakis, Michael; Keck, Wolfgang; Gray, Christopher P. (F. Hofmann-La Roche Ltd., Basel, CH-4070, Switz.). Electrophoresis, 19(11), 1980-1988 (English) 1998. CODEN: ELCTDN. ISSN: 0173-0835. Publisher: Wiley-VCH Verlag GmbH.

AB 2-D electrophoresis was applied to the global anal. of the cellular response of H. influenzae to sulfamethoxazole and trimethoprim, both inhibitors of the tetrahydrofolate synthesis. Deregulation of the synthesis rate of 118 proteins, involved in different metabolic pathways, was obsd. The regulation of the genes involved in the metab. of the amino acids Met, Thr, Ser, Gly, and Asx was investigated in detail by anal. of protein synthesis and Northern hybridization. The results suggested that the synthesis of Met biosynthetic enzymes in H. influenzae is regulated in a similar fashion as in Escherichia coli. A good correlation between the results obtained by Northern hybridization and quantification of protein synthesis was obsd. In contrast to trimethoprim, sulfamethoxazole triggered the increased synthesis of the heat shock proteins DnaK, GroEL, and GroES.

L26 ANSWER 9 OF 37 CAPLUS COPYRIGHT 2002 ACS

1998:586750 Document No. 129:312992 Reference map of the low molecular mass proteins of Haemophilus influenza. Fountoulakis, Michael; Juranville, Jean-Francois; Roeder, Daniel; Evers, Stefan; Berndt, Peter; Langen, Hanno (Preclinical Central Nervous System Research Gene Technol., F. Hoffmann-La Roche Ltd., Basel, CH-4070, Switz.). Electrophoresis, 19(10), 1819-1827 (English) 1998. CODEN: ELCTDN. ISSN: 0173-0835. Publisher: Wiley-VCH Verlag GmbH.

AB Anal. of the proteome of H. influenzae by 2-D polyacrylamide gel electrophoresis on conventional Tris-glycine gels does not usually result in efficient sepn. of the proteins in the 5-20 kDa range, which are mainly accumulated in the lower acidic and basic regions. To improve the sepn. of the low mol. mass proteins, the authors used homogeneous tricine gels of 2 urea concns. in the 2-D sepn. The tricine gel systems allowed the efficient and reproducible sepn. of the proteins of the microorganism with masses at 5-20 kDa, however, no proteins with masses <5 kDa were visualized. 80 Proteins migrating in the 5-25 kDa region were identified by matrix assisted laser desorption/ionization-mass spectrometry, of which 40 identified for the first time. The digestion of the low mass proteins often produced only few peptides, which were insufficient for confident identification by mass spectrometry. The identification was occasionally achieved by a sequential digestion with 2 proteases, trypsin, or endoproteinase Lys-C as 1st and carboxypeptidase P as 2nd enzyme. The gel system described may be useful for the efficient sepn. of low mol. mass proteins from other organisms to construct std. maps.

L26 ANSWER 10 OF 37 CAPLUS COPYRIGHT 2002 ACS

1998:489100 Document No. 129:198810 Destabilized inheritance of pSC101 and other Escherichia coli plasmids by DpiA, a novel two-component system regulator. Ingmer, Hanne; Miller, Christine A.; Cohen, Stanley N. (Department of Genetics, Stanford University School of Medicine, Stanford, CA, 94305-5120, USA). Mol. Microbiol., 29(1), 49-59 (English) 1998.

CODEN: MOMIEE. ISSN: 0950-382X. Publisher: Blackwell Science Ltd..

AB We identified a gene (dpiA, destabilizer of plasmid inheritance) which, when overexpressed in Escherichia coli, destabilizes the inheritance of pSC101 and other iteron-contg. plasmids as disparate as mini-F and RK6 but not the inheritance of P1, RSF1010 and ColD. These effects of DpiA, which functions like an effector protein for a previously undescribed two-component signal transduction system, were reduced by mutations known to promote pSC101 replication and partitioning. DpiB, a gene encoding the putative histidine kinase of this two-component system, is located immediately 5' to dpiA and adjacent to a DpiA-induced target promoter that transcribes genes having homol. to ***citrate*** ***lyase*** operon genes, citC, citD and citE, of ***Klebsiella*** ***pneumoniae*** . Disruption of dpiB reversed or reduced the effect of DpiA overprodn. on pSC101 inheritance. A second DpiA target, the promoter for a gene (appY) implicated in E. coli's response to anaerobiosis, is repressed by DpiA. A mutation in dpiA at a site commonly conserved and phosphorylated in two-component system effector proteins abolished the effects of DpiA overprodn. on pSC101 inheritance and neg. regulation of appY expression. Our findings suggest a possible mechanism by which environmental and/or cellular stimuli may influence plasmid inheritance.

L26 ANSWER 11 OF 37 CAPLUS COPYRIGHT 2002 ACS

1998:281891 Document No. 129:78245 ***Citrate*** ***lyases*** of lactic acid bacteria. Bekal, Sadja; Divies, Charles; Prevost, Herve (Laboratoire de Microbiologie U.A. Inra, Ensbana, Universite de Bourgogne, Dijon, 21000, Fr.). Lait, 78(1), 3-10 (English) 1998. CODEN: LAITAG. ISSN: 0023-7302. Publisher: Editions Scientifiques et Medicales Elsevier.

AB A review with 37 refs. on the structure and regulation of ***citrate*** ***lyase*** in lactic acid bacteria is presented in comparison to the enzymes characterized in others micro-organisms. The genetic organization of genes encoding proteins involved in ***citrate*** ***lyase*** activity of ***Leuconostoc*** ***mesenteroides*** is described.

L26 ANSWER 12 OF 37 CAPLUS COPYRIGHT 2002 ACS

1998:192747 Document No. 128:292413 Large-scale identification of proteins of ***Haemophilus*** ***influenzae*** by amino acid composition analysis. Fountoulakis, Michael; Juranville, Jean Francois; Berndt, Peter (F. Hoffmann-La Roche Ltd., Basel, CH-4070, Switz.). Electrophoresis, 18(15), 2968-2977 (English) 1997. CODEN: ELCTDN. ISSN: 0173-0835. Publisher: Wiley-VCH Verlag GmbH.

AB Two-dimensional protein maps of microorganisms are useful tools for elucidation and detection of target proteins, a process essential in the development of new pharmaceutical products. The authors applied amino acid compn. anal., following sepn. by two-dimensional gel electrophoresis, for large-scale identification of proteins of ***Haemophilus*** ***influenzae*** . H. influenzae is a bacterium of pharmaceutical interest of which the entire genome, comprising approx. 1700 open reading frames, has been sequenced. For amino acid anal., the authors used both precolumn derivatization of amino acids followed by reversed-phase chromatog. of the derivatized residues and post-column derivatization of the residues previously sepd. on an ion exchanger. The compn. analyses derived from both methods allowed the identification of 110 protein spots. The proteins were identified using the AACompIdent software on the ExPASy server accessible via the World Wide Web with a success rate of 52%. In some cases, introduction of the anal. data of 12 residues was sufficient for a correct identification. Proteins which contained an unusually high percentage of one residue could be unambiguously identified. Amino acid compn. anal. proved to be an error-robust, efficient method for protein identification. The method can be practically established in every biochem. lab. and, complementary to mass spectrometry, represents an important anal. tool for the mapping of the proteomes of organisms of interest.

L26 ANSWER 13 OF 37 CAPLUS COPYRIGHT 2002 ACS

1998:65324 Document No. 128:241045 Purification of ***Leuconostoc*** ***mesenteroides*** ***citrate*** ***lyase*** and cloning and characterization of the citCDEFG gene cluster. Bekal, Sadjia; Van Beeumen, Jozef; Samyn, Bart; Garmyn, Dominique; Henini, Samia; Davies, Charles; Prevost, Herve (Laboratoire de Microbiologie, UA INRA, ENS.BANA, Universite de Bourgogne, Dijon, 21 000, Fr.). J. Bacteriol., 180(3), 647-654 (English) 1998. CODEN: JOBAAY. ISSN: 0021-9193. Publisher:

American Society for Microbiology.

AB A ***citrate*** ***lyase*** (EC 4.1.3.6) was purified 25-fold from ***Leuconostoc*** ***mesenteroides*** and was shown to contain three subunits. The first 42 amino acids of the .beta. subunit were identified, as well as an internal peptide sequence spanning some 20 amino acids into the .alpha. subunit. Using degenerated primers from these sequences, the authors amplified a 1.2-kb DNA fragment by PCR from ***Leuconostoc*** ***mesenteroides*** subsp. cremoris. This fragment was used as a probe for screening a Leuconostoc genomic bank to identify the structural genes. The 2.7-kb gene cluster encoding ***citrate*** ***lyase*** of L. mesenteroides is organized in three open reading frames, citD, citE, and citF, encoding, resp., the three ***citrate*** ***lyase*** subunits .gamma. (acyl carrier protein [ACP]), .beta. (citryl-S-ACP lyase; EC 4.1.3.34) and .alpha. (citrate:acetyl-ACP-transferase; EC 2.8.3.10). The gene (citC) encoding the ***citrate*** ***lyase*** ligase (EC 6.2.1.22) was localized in the region upstream of citD. Protein comparisons show similarities with the ***citrate*** ***lyase*** ligase and ***citrate*** ***lyase*** of ***Klebsiella*** ***pneumoniae*** and ***Haemophilus*** ***influenzae*** . Downstream of the ***citrate*** ***lyase*** cluster, a 1.4-kb open reading frame encoding a 52-kDa protein was found. The deduced protein is similar to CitG of the other bacteria, and its function remains unknown. Expression of the citCDEFG gene cluster in Escherichia coli led to the detection of a ***citrate*** ***lyase*** activity only in the presence of acetyl CoA, which is a structural analog of the prosthetic group. This shows that the acetyl-ACP group of the ***citrate*** ***lyase*** form in E. coli is not complete or not linked to the protein.

L26 ANSWER 14 OF 37 CAPLUS COPYRIGHT 2002 ACS

1997:584258 Document No. 127:259159 Identifying the major proteome components of ***Haemophilus*** ***influenzae*** type-strain NCTC 8143. Link, Andrew J.; Hays, Lara G.; Carmack, Edwin B.; Yates, John R., III (Dep. Molecular Biotechnology, Washington Univ., Seattle, WA, 98195, USA). Electrophoresis, 18(8), 1314-1334 (English) 1997. CODEN: ELCTDN. ISSN: 0173-0835. Publisher: Wiley-VCH.

AB With the completion of the H. influenzae Rd genomic sequence, the identity is known of most of the theor. proteins in the proteome of this bacterium. However, the most abundant components of the actual proteome are unknown. Using mass spectrometry and 2-dimensional gel electrophoresis (2-DE), the most abundant proteins were sequenced and analyzed obsd. in the ATCC ref. strain of H. influenzae, NCTC 8143 (303 of 400 Coomassie-stained 2-DE spots). To automate the identification of 2-DE spots, a liq. autosampler was coupled to a microcolumn liq. chromatog. electrospray ionization tandem mass spectrometer capable of identifying 22 spots per day. From the 303 sequenced spots, 263 unique proteins were identified . Most of the abundant proteins lie in an isoelec. point range of pH 4-7 and a mol. mass range of 10-100 kDa. Of the obsd. proteins, the most abundant is the outer membrane protein P2. Based on variety and abundance, proteins involved in energy metab. and macromol. synthesis are the dominant classes of proteins. Unexpectedly, tryptophanase was identified as a highly abundant protein in the strain NCTC 8143 whose sequence is not present in the genome of the Rd strain. By searching the tandem mass spectra against the translated genomic sequence, several proteins were identified which were not annotated in the genomic sequence. Surprisingly, 22% of the identified 2-DE spots represent isoforms in which gene products with the same primary sequence have different obsd. pI and Mr, indicating that these proteins are post-translationally processed. Although most proteins' predicted and obsd. isoelec. points and mol. masses show reasonable concordance, the obsd. values for several proteins deviate from the predicted values. These anomalies may represent either highly processed proteins or misinterpretations of the genomic sequence. Using the technol. developed in this project, the protein expression of other strains of H. influenzae grown under different environmental conditions can be compared to identify differences in their proteomes.

L26 ANSWER 15 OF 37 CAPLUS COPYRIGHT 2002 ACS

1997:497785 Document No. 127:106404 Enzymic and genetic basis for bacterial growth on malonate. Dimroth, Peter; Hilbi, Hubert (Mikrobiologisches Inst., Eidgenossische Technische Hochschule, Zurich, CH-8092, Switz.). Mol. Microbiol., 25(1), 3-10 (English) 1997. CODEN: MOMIEE. ISSN:

0950-382X. Publisher: Blackwell.

AB A review with 30 refs. Various bacteria are able to grow aerobically or anaerobically on malonate as sole source of carbon and energy. Independent of the mechanism for energy conservation, the decarboxylation of malonate is the key reaction in the decompn. of this compd. To achieve malonate decarboxylation under physiol. conditions, the substrate must be converted into an activated (thioester) deriv. We report here on the malonate decarboxylases of Malonomonas rubra and ***Klebsiella*** ***pneumoniae*** . These enzymes perform an interesting substrate activation mechanism by generating a malonyl-S-enzyme involves and activation module that comprises the acetylation of a specific thiol group of an acyl carrier protein (ACP) and the transfer of the ACP moiety to malonate, yielding malonyl-S-ACP and acetate. The malonyl-S-ACP is subsequently decarboxylated with regeneration of the acetyl-ACP. The malonate activation mechanism is related to the activation of citrate by ***citrate*** ***lyase*** . The relationship extends to the identical 2'-(5"-phosphoribosyl)-s'-dephospho-CoA thiol cofactor that is bound covalently to the corresponding ACP subunit. In ***Klebsiella*** ***pneumoniae*** , malonate is decarboxylated by a water-sol. enzyme complex. In the anaerobic bacterium Malonomonas rubra, malonate decarboxylation is catalyzed by a set of water-sol. as well as membrane bound enzymes that function together in converting the free energy of the decarboxylation reaction into .DELTA..mu.Na+. Therefore, this malonate decarboxylase includes a biotin carrier protein that accepts the CO2 moiety from malonyl-S-ACP and delivers it to a membrane-bound decarboxylase acting as a Na+ pump. Genes encoding the individual protein components that perform the decarboxylation of malonate in K. pneumoniae or M. rubra have been identified within the mdc and mad gene clusters resp. The function of most of the derived proteins could be envisaged from sequence similarities with proteins of known functions. The genetic evidence firmly supports the idea that malonate decarboxylation is carried out by the two different decarboxylases, as deduced from the biochem. studies of the enzymes.

L26 ANSWER 16 OF 37 CAPLUS COPYRIGHT 2002 ACS

1997:197986 Document No. 126:314535 Anaerobic citrate metabolism and its regulation in enterobacteria. Bott, Michael (Mikrobiologisches Inst., Eidgenossische Technische Hochschule Zurich, Zurich, CH-8092, Switz.). Arch. Microbiol., 167(2+3), 78-88 (English) 1997. CODEN: AMICCW. ISSN: 0302-8933. Publisher: Springer.

AB A review with many refs. Several species of enterobacteria are able to utilize citrate as C and energy source. Under oxic conditions in the presence of a functional tricarboxylic acid cycle, growth on this compd. solely depends on an appropriate transport system. During anaerobiosis, when 2-oxoglutarate dehydrogenase is repressed, some species such as ***Klebsiella*** ***pneumoniae*** and Salmonella typhimurium, but not Escherichia coli, are capable of growth on citrate by a Na+-dependent pathway forming acetate, formate, and CO2 as products. During the last decade, several novel features assocd. with this type of fermn. were discovered in K. pneumoniae. The biotin protein oxaloacetate decarboxylase, one of the key enzymes of the pathway besides ***citrate*** ***lyase*** , is a Na+ pump. Recently it has been shown that the proton required for the decarboxylation of carboxybiotin is taken up from the side to which Na+ ions are pumped, and a membrane-embedded aspartate residue that is probably involved both in Na+ and in H+ transport was identified. The Na+ gradient established by oxaloacetate decarboxylase drives citrate uptake via CitS, a homodimeric carrier protein with a simultaneous-type reaction mechanism, and NADH formation by reversed electron transfer involving formate dehydrogenase, quinone, and a Na+-dependent NADH:quinone oxidoreductase. All enzymes specifically required for citrate fermn. are induced under anoxic conditions in the presence of citrate and Na+ ions. The corresponding genes form a cluster on the chromosome and are organized as two divergently transcribed operons. Their coordinate expression is dependent on a two-component system consisting of the sensor kinase CitA and the response regulator CitB. The citAB genes are part of the cluster and are pos. auto-regulated. In addn. to CitA/CitB, the cAMP receptor protein (Crp) is involved in the regulation of the citrate fermn. enzymes, subjecting them to catabolite repression.

L26 ANSWER 17 OF 37 CAPLUS COPYRIGHT 2002 ACS

1996:661155 Document No. 125:296851 The citrate metabolic pathway in ***Leuconostoc*** ***mesenteroides*** : expression, amino acid synthesis, and .alpha.-ketocarboxylate transport. Marty-Teysset, Claire; Lolkema, Juke S.; Schmitt, Philippe; Divies, Charles; Konings, Wil N. (Dep. Microbiol., Univ. Groningen, Haren, 9751NN, Neth.). J. Bacteriol., 178(21), 6209-6215 (English) 1996. CODEN: JOBAAY. ISSN: 0021-9193.

AB Citrate metab. in ***Leuconostoc*** ***mesenteroides*** subspecies mesenteroides is assocd. with the generation of a proton motive force by a secondary mechanism. The pathway consists of four steps: (i)uptake of citrate, (ii) splitting of citrate into acetate and oxaloacetate, (iii) pyruvate formation by decarboxylation of oxaloacetate, and (i.v.) redn. of pyruvate to lactate. Studies of citrate uptake and metab. in resting cells of L. mesenteroides grown in the presence or absence of citrate show that the citrate transporter CitP and ***citrate*** ***lyase*** are constitutively expressed. On the other hand, oxaloacetate decarboxylase is under stringent control of the citrate in the medium and is not expressed in its absence, thereby blocking the pathway at the level of oxaloacetate. Under those conditions, the pathway is completely directed towards the formation of aspartate, which is formed from oxaloacetate by transaminase activity. The data indicate a role for citrate metab. in amino acid biosynthesis. Internalized radiolabeled aspartate produced from citrate metab. could be chased from the cells by addn. of the amino acid precursors oxaloacetate, pyruvate, .alpha.-ketoglutarate, and .alpha.-ketoisocaproate to the cells, indicating a broad specificity of the transamination reaction. The .alpha.-ketocarboxylates are readily transported across the cytoplasmic membrane. .alpha.-Ketoglutarate uptake in resting cells of L. mesenteroides was dependent upon the presence of an energy source and was inhibited by inhibition of the proton motive force generating F0F1 ATPase and by selective dissipation of the membrane potential and the transmembrane pH gradient. It is concluded that in L. mesenteroides .alpha.-ketoglutarate is transported via a secondary transporter that may be a general .alpha.-ketocarboxylate carrier.

L26 ANSWER 18 OF 37 CAPLUS COPYRIGHT 2002 ACS

1996:184607 Document No. 124:254213 The acyl carrier protein of malonate decarboxylase of Malonomonas rubra contains 2'-(5''-phosphoribosyl)-3'-dephosphocoenzyme A as a prosthetic group. Berg, Michael; Hilbi, Hubert; Dimroth, Peter (Mikrobiologisches Institut, ETH-Zentrum, Zurich, CH-8092, Switz.). Biochemistry, 35(15), 4689-96 (English) 1996. CODEN: BICHAW. ISSN: 0006-2960.

AB Malonate decarboxylase of Malonomonas rubra is composed of sol. and membrane-bound components and contains an acetyl residue that is essential for catalytic activity. Upon incubation with hydroxylamine, the acetyl residue is removed, forming an inactive thiol enzyme, which is reactivated by acetylation with ATP, acetate, and a specific ligase. After incubation of the thiol enzyme with iodoacetate in the presence of excess dithioerythritol, the prosthetic group thiol residue was carboxymethylated and reactivation by acetylation was impaired. Radioactive labeling with [1-14C]iodoacetate revealed the site of carboxymethylation on a distinct cytoplasmic protein with the apparent mol. mass of 14 000 Da. The same protein was specifically labeled by enzymic acetylation of the thiol enzyme with [1-14C]acetate and ATP. Malonate decarboxylation by [14C]acetyl malonate decarboxylase resulted in the release of the radioactive acetyl residue from the enzyme, indicating that this acetyl residue is exchanged for a malonyl residue during catalysis. The acyl carrier protein has been purified as its [14C]carboxymethylated deriv. to apparent homogeneity. The prosthetic group of the acyl carrier protein was isolated after alk. hydrolysis, and its chem. structure was identified by high-performance liq. chromatog. (HPLC) with the corresponding compd. from ***citrate*** ***lyase*** from ***Klebsiella*** ***pneumoniae*** as ref. and by mass spectrometry. Malonate decarboxylase was found to carry the same prosthetic group as ***citrate*** ***lyase*** , i.e. 2'-(5''-phosphoribosyl)-3'-dephospho-CoA.

L26 ANSWER 19 OF 37 CAPLUS COPYRIGHT 2002 ACS

1995:983804 Document No. 124:78105 Regulation of anaerobic citrate metabolism in ***Klebsiella*** ***pneumoniae*** . Bott, Michael; Meyer, Margareta; Dimroth, Peter (Microbiologisches Inst., Zurich, CH-8092, Switz.). Mol. Microbiol., 18(3), 533-46 (English) 1995. CODEN:

MOMIEE. ISSN: 0950-382X.

AB Three enzymes are specifically required for uptake and catabolism of citrate by ***Klebsiella*** ***pneumoniae*** under anaerobic conditions: a Na+-dependent citrate carrier (CitS), ***citrate*** ***lyase*** (CitDEF), and the Na+ pump oxaloacetate decarboxylase (OadGAB). The corresponding genes are clustered on the chromosomes, with the citCDEFG genes located upstream and divergent to the citCDEFG genes located upstream and divergent to the citS-oadGAB genes. We found that expression of citS from its native promoter in Escherichia coli requires the DNA region downstream of oadB. Nucleotide sequence anal. of this region revealed the presence of two adjacent genes, citA and citB. By sequence similarity, the predicted CitA and CitB proteins were identified as members of the two-component regulatory systems. The sensor kinase CitA contained, in the N-terminal half, two putative transmembrane helixes which enclosed a presumably periplasmic domain of about 130 amino acids. The C-terminal half of the response regulator CitB harbored a helix-turn-helix motif typical of DNA-binding proteins. K. pneumoniae citB null mutants were unable to grow anaerobically with citrate as the sole carbon and energy source (Cit- phenotype). When cultivated anaerobically with citrate plus glycerol, all of the citrate-specific fermn. enzymes were synthesized in the wild type, but not in the citB mutants. This showed that citS, oadGAB and citDEF required the CitB protein for expression and therefore are part of a regulon. In the wild type synthesis of CitS, oxaloacetate decarboxylase and ***citrate*** ***lyase*** was dependent on the presence of citrate, sodium ions and a low oxygen tension. In a citA null mutant which expressed citB constitutively at high levels, none of these signals was required for the formation of the citrate fermn. enzymes. This result suggested that citrate, Na+, and oxygen exerted their regulatory effects via the CitA/CitB system. In the presence these signals, the citAB gene products induced their own synthesis. The pos. autoregulation occurred via co-transcription of citAB with citS and oadGAB.

L26 ANSWER 20 OF 37 CAPLUS COPYRIGHT 2002 ACS

1995:173252 Document No. 122:48089 ***Klebsiella*** ***pneumoniae*** genes for ***citrate*** ***lyase*** and ***citrate*** ***lyase*** ligase: localization, sequencing, and expression. Bott, Michael; Dimroth, Peter (Mikrobiologisches Inst., Eidgenoessische Technische Hochschule, Zurich, CH-8092, Switz.). Mol. Microbiol., 14(2), 347-56 (English) 1994. CODEN: MOMIEE. ISSN: 0950-382X.

AB In the course of studies on anaerobic citrate metab., in ***Klebsiella*** ***pneumoniae*** , the DNA region upstream of the gene for the sodium-dependent citrate carrier (citS) was investigated. Nucleotide sequence anal. revealed a cluster of five new genes that were oriented inversely to citS and probably form an operon. The genes were named citCDEFG. Based on known protein sequence data, the gene products derived from citD, citE and citF could be identified as the .gamma.-, .beta.-, and .alpha.-subunits of ***citrate*** ***lyase*** , resp. This enzyme catalyzes the cleavage of citrate to oxaloacetate and acetate. The gene product derived from citC (calcd. Mr 38 476) exhibited no obvious similarity to other proteins. In the presence of acetate and ATP, cell exts. from a citC-expressing Escherichia coli strain were able to reactivate purified ***citrate*** ***lyase*** from K. pneumoniae that had been inactivated by chem. deacetylation of the prosthetic group. This represents 5-phosphoribosyl-dephospho-acetyl-CoA which is covalently bound to serine-14 of the acyl carrier protein (.gamma.-subunit). CitC was thus identified as acetate:SH- ***citrate*** ***lyase*** ligase. The function of the gene product derived from cite (Mr 32 645) has not yet been identified. Expression of the citCDEFG gene cluster in E. coli led to the formation of ***citrate*** ***lyase*** which was active only in the presence of acetyl-CoA, a compd. known to substitute for the prosthetic group. These and other data strongly indicated that the enzyme synthesized in e. coli lacked its prosthetic group. Thus, addnl. genes besides citCDEFG appear to be required for the formation of holo- ***citrate*** ***lyase*** .

L26 ANSWER 21 OF 37 CAPLUS COPYRIGHT 2002 ACS

1992:546969 Document No. 117:146969 Effect of varying citrate levels on C4 compound formation and on enzyme levels in ***Leuconostoc*** ***mesenteroides*** subsp. cremoris grown in continuous culture. Schmitt, P.; Divies, C. (Ec. Natl. Super. Biol. Appl. Nutr. Aliment.,

Univ. Bourgogne 1, Dijon, 21000, Fr.). Appl. Microbiol. Biotechnol., 37(4), 426-30 (English) 1992. CODEN: AMBIDG. ISSN: 0175-7598.

AB The effects of citrate on diacetyl, acetoin and 2,3-butylene glycol (2,3-BG) prodn. by ***Leuconostoc*** ***mesenteroides*** subsp. cremoris grown in continuous culture at pH 5.2 were studied. In glucose alone end-product prodn. agreed with the theor. stoichiometry. In the presence of citrate, lactate and acetate prodn. was higher than the theor. stoichiometry from glucose. Lactate prodn. was const. when the initial citrate concn. was increased whereas ethanol prodn. strongly decreased. In the absence of citrate, ***citrate*** ***lyase*** (CL) exhibited weak activity. Diacetyl reductase (DR) and acetoin reductase (AR) exhibited basal activity. When varying citrate concns. ranging from 10 to 75 mM were added to glucose broth, DR, AR, lactate dehydrogenase, NADH oxidase and alc. dehydrogenase decreased as the initial citrate concns. increased suggesting that they were partly repressed by citrate. In contrast, CL increased and the specific citrate utilization rate also increased in the same way, indicating no satn. of the first step of citrate metab. Acetate kinase (AK) was slightly higher in the presence of citrate and increased when the initial citrate concn. increased. This result was correlated with an increase of acetate from the acetyl phosphate pathway. More ATP was produced in the presence of citrate, which could explain the increase in biomass formation. Citrate bioconversion into diacetyl, acetoin and 2,3-BG increased as the initial citrate increased.

L26 ANSWER 22 OF 37 CAPLUS COPYRIGHT 2002 ACS

1991:554721 Document No. 115:154721 Characterization of a citrate-negative mutant of ***Leuconostoc*** ***mesenteroides*** subsp. mesenteroides: metabolic and plasmidic properties [Erratum to document cited in CA114(19):181893j]. Lin, J.; Schmitt, P.; Divies, C. (Dep. Microbiol. Biotechnol., Ec. Natl. Super. Biol. Appl. Nutr. Aliment., Dijon, F-21000, Fr.). Appl. Microbiol. Biotechnol., 35(3), 420 (English) 1991. CODEN: AMBIDG. ISSN: 0175-7598.

AB Errors in Table 2 have been cor. A cor. Figure 2 has been provided. The errors were not reflected in the abstr. or the index entries.

L26 ANSWER 23 OF 37 CAPLUS COPYRIGHT 2002 ACS

1991:181893 Document No. 114:181893 Characterization of a citrate-negative mutant of ***Leuconostoc*** ***mesenteroides*** subsp. mesenteroides: metabolic and plasmidic properties. Lin, J.; Schmitt, P.; Divies, C. (Dep. Microbiol. Biotechnol., Ec. Natl. Super. Biol. Appl. Nutr. Aliment., Dijon, F-21000, Fr.). Appl. Microbiol. Biotechnol., 34(5), 628-31 (English) 1991. CODEN: AMBIDG. ISSN: 0175-7598.

AB Comparison of the parental strain of the L. mesenteroides subsp. mesenteroides (19D) and its citrate-neg. mutant, which has lost a 22-kb plasmid, has confirmed the energetic role of citrate. Fermn. balance anal. showed that citrate led to a change in heterolactic fermn. from glucose. High levels of enzyme activity in both mutant and parental strains were found for NADH oxidase, lactate dehydrogenase, acetate kinase, alc. dehydrogenase, diacetyl reductase and acetoin reductase, although NADH oxidase, alc. dehydrogenase, diacetyl reductase, and acetoin reductase were partly repressed by citrate. All these enzymes studied were not plasmid-linked. In the parental strain, ***citrate*** ***lyase*** was induced by citrate. No ***citrate*** ***lyase*** activity was found in the citrate-neg. mutant grown in presence of citrate, but this does not provide evidence that ***citrate*** ***lyase*** is linked to the 22-kb plasmid.

L26 ANSWER 24 OF 37 CAPLUS COPYRIGHT 2002 ACS

1991:20074 Document No. 114:20074 ***Citrate*** ***lyase*** from ***Klebsiella*** ***pneumoniae*** . The complete primary structure of the acyl lyase subunit. Hupperich, Martin; Henschen, Agnes; Eggerer, Hermann (Inst. Physiol. Chem., Tech. Univ. Muenchen, Munich, D-8000/40, Fed. Rep. Ger.). Eur. J. Biochem., 192(1), 161-6 (English) 1990. CODEN: EJBCAI. ISSN: 0014-2956.

AB The primary structure of the .beta.-subunit (acyl lyase subunit) of ***citrate*** ***lyase*** from K. pneumoniae (ATCC 13882) was detd. with protein chem. methods. The polypeptide chain consists of 289 amino acid residues and has a mol. mass of 31,352 Da. The two half-cystine residues of the subunit are present as cysteines and not involved in disulfide bridges. The sequence shows no homol. to known sequences of

proteins or nucleic acids.

L26 ANSWER 25 OF 37 CAPLUS COPYRIGHT 2002 ACS
1987:529918 Document No. 107:129918 Electron microscopical investigation of ***citrate*** ***lyase*** single molecules. Ihle, Ekkehard; Schramm, Hans J. (Max-Planck-Inst. Biochem., Martinsried, D-8033, Fed. Rep. Ger.). Biol. Chem. Hoppe-Seyler, 368(7), 787-93 (English) 1987. CODEN: BCHSEI.
AB Electron micrographs of ***citrate*** ***lyase*** from Rhodopseudomonas gelatinosa and Klebsiella aerogenes revealed 2 characteristic mol. forms. The basket form and the star form were subjected to 2-dimensional image reconstruction using a technique involving averaging of superposed single mol. images after rotational correlation. A 3-dimensional image reconstruction showed that the images of these forms can be interconverted by rotation and that they therefore represent different views of the same structure.

L26 ANSWER 26 OF 37 CAPLUS COPYRIGHT 2002 ACS
1986:530511 Document No. 105:130511 Citrate transport in ***Klebsiella*** ***pneumoniae*** . Dimroth, Peter; Thomer, Anna (Inst. Physiol. Chem., Tech. Univ. Muenchen, Munich, D-8000/40, Fed. Rep. Ger.). Biol. Chem. Hoppe-Seyler, 367(8), 813-23 (English) 1986. CODEN: BCHSEI.
AB Na ions were specifically required for citrate degrdn. by suspensions of K. pneumoniae cells which had been grown anaerobically on citrate. The rate of citrate degrdn. was considerably lower than the activities of the citrate fermn. enzymes ***citrate*** ***lyase*** and oxaloacetate decarboxylase, indicating that citrate transport is rate limiting. Uptake of citrate into cells was also Na+-dependent and was accompanied by its rapid metab. so that the tricarboxylic acid was not accumulated in the cells to significant levels. The transport was stimulated less efficiently by LiCl. Li ions were cotransported with citrate into the cells. Transport and degrdn. of citrate were abolished with the uncoupler (4-(trifluoromethoxy)phenylhydrazono)propanedinitrile (CCFP). After releasing outer membrane components and periplasmic binding proteins by cold osmotic shock treatment, citrate degrdn. became also sensitive towards monensin and valinomycin. The shock procedure had no effect on the rate of citrate degrdn. indicating that the transport is not dependent on a binding protein. Citrate degrdn. and transport were independent of Na+ ions in K. pneumoniae grown aerobically on citrate and in Escherichia coli grown anaerobically on citrate plus glucose. An E. coli cit+ clone obtained by transformation of K. pneumoniae genes coding for citrate transport required Na+ specifically for aerobic growth on citrate indicating that the Na+-dependent citrate transport system is operating. Na+ and Li+ were equally effective in stimulating citrate degrdn. by cell suspensions of E. coli cit+. Citrate transport in membrane vesicles of E. coli cit+ was also Na+ dependent and was energized by the protonmotive force (.DELTA..mu.H+). Dissipation of .DELTA..mu.H+ or its components .DELTA.pH or .DELTA..psi. by ionophores either totally abolished or greatly inhibited citrate uptake. It is suggested that the systems energizing citrate transport under anaerobic conditions are provided by the outwardly directed cotransport of metabolic endproducts with protons yielding .DELTA.pH and by the decarboxylation of oxaloacetate yielding .DELTA.pHa+ and .DELTA..psi.. In citrate-fermenting K. pneumoniae an ATPase which is activated by Na+ was not found. The cells contain however a proton-translocating ATPase and a Na+/H+ antiporter in their membrane.

L26 ANSWER 27 OF 37 CAPLUS COPYRIGHT 2002 ACS
1985:521639 Document No. 103:121639 Transformation of citric acid to acetic acid, acetoin and diacetyl by wine making lactic acid bacteria. Shimazu, Yoshimi; Uehara, Mikio; Watanabe, Masazumi (Food Res. Lab., Kikkoman Corp., Noda, 278, Japan). Agric. Biol. Chem., 49(7), 2147-57 (English) 1985. CODEN: ABCHA6. ISSN: 0002-1369.
AB A decrease in citric acid [77-92-9] and increases in HOAc [64-19-7], acetoin [513-86-0], and diacetyl [431-03-8] were found in red wine and synthetic solns. after inoculation of intact cells of ***Leuconostoc*** ***mesenteroides*** lactosum. It was concluded that citric acid in wine involving malolactic fermn. is first converted by ***citrate*** ***lyase*** [***9012-83-3***] to HOAc and oxaloacetic acid [328-42-7], and the latter was transformed by decarboxylation to pyruvic acid [127-17-3] which was subsequently coverted to acetoin, diacetyl, and HOAc. Both the activities of ***citrate*** ***lyase*** and

acetoin formation from pyruvic acid in the dialyzed cell-free ext. were optimal at pH 6.0. Divalent cations activated ***citrate*** ***lyase*** . ***Citrate*** ***lyase*** was completely inhibited by EDTA, Hg2+ and Ag2+. Acetoin formation from pyruvic acid was significantly stimulated by thiamin pyrophosphate and CoCl2 and inhibited by oxaloacetic acid. Specific activities of ***citrate*** ***lyase*** and acetoin formation varied considerably among the 6 strains of malolactic bacteria examd. Some irreversible redn. of diacetyl to acetoin were found in cell-free exts. of 4 of the malolactic bacteria; the optimal pH was 6.0 for this activity in L. mesenteroides.

L26 ANSWER 28 OF 37 CAPLUS COPYRIGHT 2002 ACS
1984:508892 Document No. 101:108892 Metabolism of citric acid by the lactic acid bacteria of the malolactic fermentation of wines. Lonvaud-Funel, A.; Zmirou, C.; Larue, F. (Inst. OEnol., Univ. Bordeaux II, Talence, 33405, Fr.). Sci. Aliments, 4(Hors Ser. 3), 81-5 (French) 1984. CODEN: SCALDC. ISSN: 0240-8813.
AB Citric acid [77-92-9] metab. by the lactic acid bacteria of wine, esp. ***Leuconostoc*** ***mesenteroides*** , was studied. Citrate is converted to acetoin [513-86-0], diacetyl [431-03-8], and HOAc [64-19-7]. Wine does not contain a large amt. of citrate and regulation of bacterial ***citrate*** ***lyase*** [***9012-83-3***] by malate and glucose prevents total degrdn. of citrate, but metab. of citrate nevertheless contributes to the concn. of volatile acids and acetoin compds.

L26 ANSWER 29 OF 37 CAPLUS COPYRIGHT 2002 ACS
1983:466667 Document No. 99:66667 S-Acylated residues of the acyl-carrier protein subunit of Klebsiella aerogenes ***citrate*** ***lyase*** . Basu, Amaresh; Subramanian, Subhalakshmi; Hiremath, Leena S.; SivaRaman, Churya (Biochem. Div., Natl. Chem. Lab., Poona City, 411008, India). Biochem. Biophys. Res. Commun., 114(1), 310-17 (English) 1983. CODEN: BBRCA9. ISSN: 0006-291X.
AB Oxidn. of the isolated deacetyl-[acyl carrier protein] [ACP] subunit of ***citrate*** ***lyase*** from K. aerogenes with the Cu2+-o-phenanthroline complex leads exclusively to intrapeptide disulfide bridge formation, indicating that the cysteamine and the cysteine residues are located in close proximity. The S-acetylation of the cysteine residue in the deacetyl-[ACP] subunit is catalyzed by a ***citrate*** ***lyase*** ligase prepn. in the presence of acetate and ATP. Reaction-inactivation of ***citrate*** ***lyase*** results in deacetylation of the S-acetylcysteamine residue of the prosthetic group, but not of the S-acylated cysteine residue in ACP.

L26 ANSWER 30 OF 37 CAPLUS COPYRIGHT 2002 ACS
1983:211981 Document No. 98:211981 Turnover and inactivation of bacterial ***citrate*** ***lyase*** with 2-fluorocitrate and 2-hydroxycitrate stereoisomers. Rokita, Steven E.; Walsh, Christopher T. (Dep. Chem., Massachusetts Inst. Technol., Cambridge, MA, 02139, USA). Biochemistry, 22(12), 2821-8 (English) 1983. CODEN: BICHAW. ISSN: 0006-2960.
AB Bacterial (Klebsiella aerogenes) ***citrate*** ***lyase*** (I) catalytically cleaves the C skeleton of the naturally occurring 2-fluorocitrate (II) isomer, (-)-erythro-II (2R,3R), with the same regiospecificity as with citrate cleavage. The C-skeleton cleavage rate of this analog is 1% of the Vmax for citrate turnover, and it binds to the enzyme with a Ki of 4.9 .mu.M, 300-fold lower than the Km of citrate. Cleavage of this analog yields oxalacetate and a fluoroacetyl form of the resting I (vs. the Ac form after citrate turnover). (-)-erythro-II inactivates I .apprx.500-fold more frequently than citrate, producing the deacetylated, inactive form of the enzyme. (+)-erythro-II (2S,3S) is also cleaved very slowly (0.05% of the Vmax for citrate), but does not cause measurable enzyme inactivation. Even when the turnover rate of I is reduced 2000-fold as above, the regiospecificity of processing remains the same as that of citrate, yielding .beta.-fluorooxalacetate and the Ac form of resting I. Among the 4 diastereomers of 2-hydroxycitrate (III), 3 forms, (+)-threo-III, (-)-threo-III, and (-)-erythro-III, are catalytically cleaved by I. The regiospecificity of processing in all cases is invariant and identical with that of citrate cleavage. (-)-erythro-III (2R,3S) and (-3)-threo-III (2S,3S) yield oxalacetate and the glycolyl form of resting I in the presence of either Zn2+ or Mg2+. (+)-threo-III (2R,3R) yields 3-hydroxyoxalacetate and the Ac form of I if

Mg2+ is present, but no catalytic activity is evident in the presence of Zn2+. The (+)-threo and (-)-erythro isomers inactivate I with partition ratios of <100 catalytic cycles per enzyme inactivation. (+)-erythro-III (2S,3R) appears to be a completely efficient turnover-dependent inactivator with a kinact of 0.68-1.1 min-1 depending on the metal ion present. This isomer effects net hydrolysis of the active, Ac form of the enzyme to a catalytically inactive form without producing any detectable C-skeleton cleavage. Enzyme inactivation induced by all of the above citrate analogs may follow a common path. The hydrolysis of a catalytic intermediate, the mixed-acid anhydride, could be the sole cause of enzyme inactivation.

L26 ANSWER 31 OF 37 CAPLUS COPYRIGHT 2002 ACS
1983:139742 Document No. 98:139742 The presence of essential arginine residues at the active sites of ***citrate*** ***lyase*** complex from Klebsiella aerogenes. Subramanian, Subhalakshmi; Basu, Amaresh; SivaRaman, Churya (Biochem. Div., Natl. Chem. Lab., Poona City, 411 008, India). Biochem. Biophys. Res. Commun., 111(2), 490-7 (English) 1983. CODEN: BBRCA9. ISSN: 0006-291X.
AB The acyltransferase and acyl-lyase activities of the K. aerogenes ***citrate*** ***lyase*** complex were inactivated by the arginine-specific reagents, phenylglyoxal and 2,3-butanedione, the former reagent being the more potent inhibitor. Citrate and (3S)-citryl-CoA protected the transferase activity, whereas acetyl-CoA markedly enhanced the rate of the inactivation. (3S)-Citryl-CoA protected the lyase subunit in the complex from inactivation. The kinetics of inactivation suggest the involvement of a single arginine residue at each of the active sites of the transferase and of the lyase subunits.

L26 ANSWER 32 OF 37 CAPLUS COPYRIGHT 2002 ACS
1982:506179 Document No. 97:106179 Photoaffinity labeling of Klebsiella aerogenes ***citrate*** ***lyase*** by p-azidobenzoyl-CoA. Basu, Amaresh; Subramanian, Subhalakshmi; SivaRaman, Churya (Biochem. Div., Natl. Chem. Lab., Poona, 411 008, India). Biochemistry, 21(18), 4434-7 (English) 1982. CODEN: BICHAW. ISSN: 0006-2960.
AB p-Azidobenzoyl-CoA (I) functions as a linear competitive inhibitor for (3S)-citryl-CoA (II) in the ***citrate*** ***lyase*** (EC 4.1.3.6)(III) reaction catalyzed by the K. aerogenes deacetyl-III complex (Ki = 80 .mu.M; II, Km = 67 .mu.M). Inactivation is irreversible on photolysis of I in the presence of the deacetyl-III complex. Mg2+ is not required for the inactivation. Inactivation is blocked by II in the presence of EDTA. I has no effect on the acetyl-CoA-citrate CoA-transferase activity both of the deacetyl-III complex and of its isolated transferase subunit. The stoichiometry of the CoA ester binding has been investigated by the use of I-14C as a photoaffinity reagent. The labeling is exclusively on the III .beta. subunit of the multienzyme complex.

L26 ANSWER 33 OF 37 CAPLUS COPYRIGHT 2002 ACS
1979:606289 Document No. 91:206289 Cooperative binding of manganese to ***citrate*** ***lyase*** from Klebsiella aerogenes. Sivaraman, H.; Sivaraman, C. (Biochem. Div., Natl. Chem. Lab., Poona, 411 008, India). FEBS Lett., 105(2), 267-70 (English) 1979. CODEN: FEBLAL. ISSN: 0014-5793.
AB The cooperative binding of Mn2+ to ***citrate*** ***lyase*** (I) (EC 4.1.3.6) of K. aerogenes was studied by equil. dialysis using 54MnCl2. The satn. curve of Mn2+ binding, the Hill plot, and Scatchard plot are shown. I binding sites for Mn2+ were satd. at .gtorsim.1 .times. 10-3M Mn2+ with 18 g atoms of Mn2+ bound/mol enzyme. The satn. plot was sigmoidal, diagnostic of pos. cooperativity. The Hill coeff., 2.27, also indicated significant pos. cooperativity, as did the max. in the Scatchard plot. The microscopic dissocn. const., Kd,n of the Mn2+-I complex for the last binding step was calcd. as 4.5 .times. 10-5M at satn. Mn2+ binding evidently involves a conformation change in the enzyme.

L26 ANSWER 34 OF 37 CAPLUS COPYRIGHT 2002 ACS
1979:147676 Document No. 90:147676 The configuration and location of the ribosidic linkage in the prosthetic group of ***citrate*** ***lyase*** (Klebsiella aerogenes). Oppenheimer, Norman J.; Singh, Manoranjan; Sweeley, Charles C.; Sung, S. J.; Srere, Paul A. (Dep. Pharm. Chem., Univ. California, San Francisco, Calif., USA). J. Biol. Chem.,

254(4), 1000-2 (English) 1979. CODEN: JBCHA3. ISSN: 0021-9258.
AB The structure of the prosthetic group of ***citrate*** ***lyase*** of K. aerogenes was studied by NMR and mass spectrometry. The spectra at 360 MHz of the nucleoside moiety, 2'-ribosyladenosine, showed the absence of 2'-hydroxyl proton, thus confirming the 2'-position as the site of attachment of the 2nd ribose moiety to the dephospho-CoA. This glycosidic linkage was found to be .alpha.(1''.fwdarw.2') and was identical to that of poly(ADP-ribose). Studies of permethylation products by mass spectrometry supported the above conclusion regarding the location of the ribosidic linkage.

L26 ANSWER 35 OF 37 CAPLUS COPYRIGHT 2002 ACS
1977:68195 Document No. 86:68195 Energy metabolism of some representatives of the Haemophilus group. Hollaender, R. (Hyg.-Inst., Philipps-Univ., Marburg/Lahn, Ger.). Antonie van Leeuwenhoek, 42(4), 429-44 (English) 1976. CODEN: ALJMAO.
AB An addn. of glucose or lactate to H. influenzae, H. parainfluenzae, and H. aegyptius grown in a complex medium, a significant increase in cell mass was obsd. Carbohydrate degrdn. varied with the strains tested so that at the end of the exponential growth phase only small amts. of the supplements (glucose and lactate) could be detected. All strains contained the enzymes of Embden-Meyerhof-Parnas and Enter-Doudoroff pathways, hexosemonophosphate shunt, tricarboxylic acid cycle, and gluconeogenesis with an extremely high activity of malate dehydrogenase. The concns. of cytochromes varies with culture conditions and suggested different roles of cytochrome b and c in aerobic and anaerobic electron transport to fumarate.

L26 ANSWER 36 OF 37 CAPLUS COPYRIGHT 2002 ACS
1976:416316 Document No. 85:16316 Subunit and chemical composition of ***citrate*** ***lyase*** from ***Klebsiella*** ***pneumoniae*** . Singh, Manoranjan; Srere, Paul A.; Klapper, David G.; Capra, J. Donald (Pre-Clin. Sci. Unit, VA Hosp., Dallas, Tex., USA). J. Biol. Chem., 251(10), 2911-15 (English) 1976. CODEN: JBCHA3.
AB ***Citrate*** ***lyase*** from K. pneumoniae is shown by amino acid sequencing techniques to contain equimolar amts. of 3 nonidentical subunits. Together with the mol. wt. of the enzyme, this finding is interpreted to indicate that there are 6 mol. of each of these subunits per mol. of native enzyme. A partial amino acid sequence of each of the 3 subunits is presented. The pure enzyme with a specific activity of 73 units/mg of dry protein has an absorbance index (E1 cm1% at 278 nm) of 6.2. The amino acid compn. of the native enzyme is presented. In addn., the amts. of adenine, phosphate, taurine (from cysteamine), and ribose, present as a bound cofactor of unknown structure, were estd. A comparison of amino acid compns. and the partial amino acid sequences of 2 of the subunits reveals some resemblance between them.

L26 ANSWER 37 OF 37 CAPLUS COPYRIGHT 2002 ACS
1976:14503 Document No. 84:14503 Incorporation of pantothenate into ***citrate*** ***lyase*** by a pantothenateless mutant of ***Klebsiella*** ***pneumoniae*** . Singh, Manoranjan; Dempsey, Walter B.; Srere, Paul A. (Pre-Clin. Sci. Unit, VA Hosp., Dallas, Tex., USA). J. Bacteriol., 124(2), 686-92 (English) 1975. CODEN: JOBAAY.
AB A pantothenate-requiring mutant of K. pneumoniae was isolated. The mutant showed an abs. dependence on pantothenate for growth. When grown in the presence of pantothenate-14C, the mutant incorporated pantothenate-14C into ***citrate*** ***lyase*** (3.4 mol/mol of enzyme). Anal. of a double-labeled enzyme (pantothenate-14C and acetate-3H) by gel electrophoresis in Na dodecyl sulfate showed that both 3H and 14C were assocd. solely with the smallest subunit, the acyl carrier protein of ***citrate*** ***lyase*** .

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=> E BOTT M/AU
=> S E3,E5,E14,E15
             8 "BOTT M"/AU
             4 "BOTT M H P"/AU
            36 "BOTT MICHAEL"/AU
             1 "BOTT MICHAEL H"/AU
L27         49 ("BOTT M"/AU OR "BOTT M H P"/AU OR "BOTT MICHAEL"/AU OR "BOTT
               MICHAEL H"/AU)
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=> E DIMROTH P/AU
=> S E3,E4
            15 "DIMROTH P"/AU
           242 "DIMROTH PETER"/AU
L28        257 ("DIMROTH P"/AU OR "DIMROTH PETER"/AU)

=> E SCHNEIDER K/AU
=> S E3,E25
           241 "SCHNEIDER K"/AU
            15 "SCHNEIDER KARIN"/AU
L29        256 ("SCHNEIDER K"/AU OR "SCHNEIDER KARIN"/AU)

=> S L27,L28,L29
L30        546 (L27 OR L28 OR L29)

=> S L30 AND L9
L31         24 L30 AND L9

=> S L31 NOT (L19 OR L26)
L32         12 L31 NOT (L19 OR L26)

=> D 1-12 CBIB ABS

L32  ANSWER 1 OF 12  CAPLUS  COPYRIGHT 2002 ACS
1998:542210   Document No. 129:227916  The Escherichia coli citrate carrier
     CitT: a member of a novel eubacterial transporter family related to the
     2-oxoglutarate/malate translocator from spinach chloroplasts.  Pos, Klaas
     Martinus;   ***Dimroth, Peter***  ;   ***Bott, Michael***
     (Mikrobiologisches Institut, Eidgenossische Technische Hochschule Zurich,
     Zurich, CH-8092, Switz.).  J. Bacteriol., 180(16), 4160-4165 (English)
     1998.  CODEN: JOBAAY.  ISSN: 0021-9193.  Publisher: American Society for
     Microbiology.
AB   Under anoxic conditions in the presence of an oxidizable cosubstrate such
     as glucose or glycerol, Escherichia coli converts citrate to acetate and
     succinate.  Two enzymes are specifically required for the fermn. of the
     tricarboxylic acid, i.e., a citrate uptake system and   ***citrate***
       ***lyase***  .  Here we report that the open reading frame (designated
     citT) located at 13.90 min on the E. coli chromosome between rna and the
       ***citrate***     ***lyase***   genes encodes a citrate carrier.  E. coli
     transformed with a plasmid expressing citT was capable of aerobic growth
     on citrate, which provides convincing evidence for a function of CitT as a
     citrate carrier.  Transport studies with cell suspensions of the
     transformed strain indicated that CitT catalyzes a homologous exchange of
     citrate or a heterologous exchange against succinate, fumarate, or
     tartrate.  Since succinate is the end product of citrate fermn. in E.
     coli, it is likely that CitT functions in vivo as a citrate/succinate
     antiporter.  Anal. of the primary sequence showed that CitT (487 amino
     acids, 53.1 kDa) is a highly hydrophobic protein with 12 putative
     transmembrane helixes.  Sequence comparisons revealed that CitT is related
     to the 2-oxoglutarate/malate translocator (SODit1 gene product) from
     spinach chloroplasts and five bacterial gene products, none of which has
     yet been functionally characterized.  It is suggested that the E. coli
     CitT protein is a member of a novel family of eubacterial transporters
     involved in the transport of di- and tricarboxylic acids.

L32  ANSWER 2 OF 12  CAPLUS  COPYRIGHT 2002 ACS
1994:674987   Document No. 121:274987  Purification and characterization of a
     cytoplasmic enzyme component of the Na+-activated malonate decarboxylase
     system of Malonomonas rubra: acetyl-S-acyl carrier protein:malonate acyl
     carrier protein-SH transferase.  Hilbi, Hubert;   ***Dimroth, Peter***
     (Eidgenoessische Technische Hochschule, ETH-Zentrum, Zuerich, Switz.).
     Arch. Microbiol., 162(1-2), 48-56 (English) 1994.  CODEN: AMICCW.  ISSN:
     0302-8933.
AB   Malonate decarboxylation by crude exts. of Malonomonas rubra was
     specifically activated by Na+ and less efficiently by Li+ ions.  The exts.
     contained an enzyme catalyzing CoA transfer from malonyl-CoA to acetate,
     yielding acetyl CoA and malonate.  After about a 26-fold purifn. of the
     malonyl-CoA:acetate CoA transferase, an almost pure enzyme was obtd.,
     indicating that about 4% of the cellular protein consisted of the CoA
     transferase.  This abundance of the transferase is in accord with its
     proposed role as an enzyme component of the malonate decarboxylase system,
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the key enzyme of energy metab. in this organism. The apparent mol. wt. of the polypeptide was 67,000 as revealed from SDS-PAGE. A similar mol. wt. was estd. for the native transferase by gel chromatog., indicating that the enzyme exists as a monomer. Kinetic anal. of the CoA transferase yielded the following: pH-optimum at pH 5.5, an apparent Km for malonyl-CoA of 1.9 mM, for acetate of 54 mM, for acetyl-CoA for 6.9 mM, and for malonate of 0.5 mM. Malonate or citrate inhibited the enzyme with an apparent Ki of 0.4 mM and 3.0 mM, resp. The isolated CoA transferase increased the activity of malonate decarboxylase of a crude enzyme system, in which part of the endogenous CoA transferase was inactivated by borohydride, about three-fold. These results indicate that the CoA transferase functions physiol. as a component of the malonate decarboxylase system, in which it catalyzes the transfer of acyl carrier protein from acetyl acyl carrier protein and malonate to yield malonyl acyl carrier protein and acetate. Malonate is thus activated on the enzyme by exchange for the catalytically important enzyme-bound acetyl thioester residues noted previously. This type of substrate activation resembles the catalytic mechanism of ***citrate*** ***lyase*** and citramalate lyase.

L32 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2002 ACS

1978:502667 Document No. 89:102667 Amino acid sequence of ***citrate*** ***lyase*** acyl-carrier protein from Klebsiella aerogenes. Beyreuther, Konrad; Boehmer, Hildegard; ***Dimroth, Peter*** (Inst. Genet., Univ. Koeln, Cologne, Ger.). Eur. J. Biochem., 87(1), 101-10 (English) 1978. CODEN: EJBCAI. ISSN: 0014-2956.

AB The complete amino acid sequence of the acyl-carrier protein of ***citrate*** ***lyase*** from K. aerogenes was detd. by automated sequential Edman degrdns. of the protein and of peptides in a liq.-phase sequencer of Edman and a solid-phase sequencer of Laursen. The sequence composed of 78 residues, does not contain tyrosine or histidine residues. The primary structure is given. The calcd. mol. wt. of the acyl-carrier protein is 9378. The phosphoribosyl dephospho-CoA prosthetic group is bound in phosphodi ester linkage to serine-14 of the acyl-carrier protein. CD measurements indicated that ***citrate*** ***lyase*** acyl-carrier protein has a high degree of .alpha.-helical structure.

L32 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2002 ACS

1978:18209 Document No. 88:18209 Characterization of the isolated transferase subunit of ***citrate*** ***lyase*** as a CoA-transferase. Evidence against a covalent enzyme-substrate intermediate. ***Dimroth, Peter*** ; Loyal, Rosemarie; Eggerer, Hermann (Fachber. Biol. Vorklin. Med., Univ. Regensburg, Regensburg, Ger.). Eur. J. Biochem., 80(2), 479-88 (English) 1977. CODEN: EJBCAI.

AB The largest subunit of citrate lyse from Klebsiella aerogenes represents an acetylthio-[acyl carrier protein]:citrate [acyl carrier protein]-transferase but is also an acetyl-CoA:citrate CoA-transferase. The enzyme catalyzes an exchange of acetyl residues between acetylthio-[acyl carrier protein] or acetyl-CoA and acetate. The reaction occurs in the presence and, at a much lower rate, also in the absence of acetate. The citrate-independent reaction was attributed to a substitution of citrate by acetate as a substrate analog. Addnl., the isolated enzyme, in the presence of acetyl-CoA and citrate, catalyzed the formation of (3S)-citryl-CoA and is thus unequivocally a CoA-transferase. The catalytic efficiency of the enzyme in the CoA-transfer reaction is similar to that of other CoA-transferases. It was concluded that the transferase subunit of ***citrate*** ***lyase*** and the classical Co-A-transferases could belong to one family of enzymes. Attempts to demonstrate this relation were based on a comparison of the mechanism of action of the transferase subunit of ***citrate*** ***lyase*** with that established for CoA-transferases. These form enzyme-CoA and anhydride intermediates. Evidence is presented for the generation of anhydride intermediates on the transferase subunit of ***citrate*** ***lyase*** . The anhydrides are citric acetic anhydride and acetic anhydride in the acetyl exchange reactions with and without citrate, resp. In contrast to other CoA-transferases, however, the transferase-catalyzed CoA-transfer from acetyl-CoA to citrate, apparently involves no enzyme-CoA intermediate. Neither the results of several different chem. approaches nor the kinetics were compatible with the formation of this intermediate. An explanation is proposed and the consequences are discussed.

L32 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2002 ACS
1978:18208 Document No. 88:18208 Isolation and function of the subunits of citramalate lyase and formation of hybrids with the subunits of ***citrate*** ***lyase*** . ***Dimroth, Peter*** ; Buckel, Wolfgang; Loyal, Rosemarie; Eggerer, Hermann (Fachber. Biol. Vorklin. Med., Univ. Regensburg, Regensburg, Ger.). Eur. J. Biochem., 80(2), 469-77 (English) 1977. CODEN: EJBCAI.

AB The citramalate lyase complex from Clostridium tetanomorphum, composed of 6 copies of 3 different proteins, was dissocd. into its protein components and these were isolated in an enzymically active state. Citramalate lyase was reconstituted from the isolated subunits. The enzyme complex consists of an acyl carrier protein and 2 different enzymes, the .alpha. and .beta. subunits. The isolated enzymes were active if acetylthio-[acyl carrier protein] and (3S)-citramalylthio-[acyl carrier protein] were substituted for the corresponding acyl-CoA derivs. The .alpha. subunit in the presence of (3S)-citramalyl-CoA and acetate catalyzed the formation of acetyl-CoA and citramalate. The .beta. subunit catalyzed the cleavage of (3S)-citramalyl-CoA to acetyl-CoA and pyruvate. This reaction was dependent on Mg2+ and was abolished if EDTA was added in excess. It was concluded that the .alpha. subunit represents an acetylthio-[acyl carrier protein]:citramalate [acyl carrier protein]-transferase within the native complex, and the .beta. subunit a (3S)-citramalylthio-[acyl carrier protein] lyase. Results similar to those given above were previously obtained with the ***citrate*** ***lyase*** complex from Klebsiella aerogenes, but with citrate, (3S)citrylthio-[acyl carrier protein], or (3S)-citryl-CoA instead of citramalate and its corresponding derivs. Thus, not only mol. wt., subunit compn. and chem. events during substrate turnover are nearly identical in the 2 different enzyme complexes but also the functions of the individual subunits. The close relation between the 2 different enzymes from the 2 different microorganisms was confirmed by cross-reactions between subunits of each enzyme complex producing a hybrid enzyme complex.

L32 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2002 ACS
1977:401711 Document No. 87:1711 Structure of the prosthetic groups of ***citrate*** ***lyase*** and citramalate lyase. ***Dimroth,*** *** Peter*** ; Loyal, Rosemarie (Fachber. Biol. Vorklin. Med., Univ. Regensburg, Regensburg, Ger.). FEBS Lett., 76(2), 280-3 (English) 1977. CODEN: FEBLAL.

AB ***Citrate*** ***lyase*** (I) and citramalate lyase (II) both contain the same prosthetic group, covalently bound dephospho-CoA (III). In I, the linkage between III and the protein occurs through ribose 5-phosphate. This is bound in phosphodiester linkage to serine and glycosidically to the 2'- or 3'-hydroxyl group of ribose of III. The binding of III to II is probably the same, as its acyl carrier protein contains 1 mol each of phosphate and sugar in addn. to the components of III. The amino acid compn. of the acyl carrier proteins of I and II are similar, except for a markedly higher lysine content in the acyl carrier protein of II.

L32 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2002 ACS
1976:175844 Document No. 84:175844 The prosthetic group of ***citrate*** ***lyase*** acyl-carrier protein. ***Dimroth, Peter*** (Fachber. Biol. Vorklin. Med., Univ. Regensburg, Regensburg, Ger.). Eur. J. Biochem., 64(1), 269-81 (English) 1976. CODEN: EJBCAI.

AB The acyl carrier protein of ***citrate*** ***lyase*** contains adenine, phosphate, sugar, cysteamine, .beta.-alanine, and pantoic acid in molar ratio of 1:2:2:1:1:1. Peptides contg. these components in the same stoichiometric relation were isolated after proteolytic digestion of acyl carrier protein. All components were linked together in a single prosthetic group. This was released from the peptide by mild alk. hydrolysis. Under these conditions a phosphodiester bond is cleaved which links the prosthetic group to a serine residue of the peptide. Incubation of the prosthetic group-contg. peptide with phosphodiesterase I yielded 4'-phosphopantetheine and adenylic acid. The 5'-AMP was not free but was substituted by presumably an acidic sugar residue, which was released by mild acid hydrolysis yielding free 5'-AMP. Thus, the prosthetic group of ***citrate*** ***lyase*** acyl carrier protein consists of a substituted isomeric dephospho-CoA. This is bound to the protein by the 5'-phosphate group of adenylic acid. The 4'-phosphopantetheine residue is bound by a pohosphodiester linkage to the 2' or 3' position of ribose and

the remaining OH group of ribose is substituted with presumably an acidic sugar residue. The structural similarities of this prothetic group and CoA are discussed and related to the catalytic properties of ***citrate*** ***lyase*** .

L32 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2002 ACS
1976:1721 Document No. 84:1721 Isolation of subunits of ***citrate*** ***lyase*** and characterization of their function in the enzyme complex. ***Dimroth, P.*** ; Eggerer, H. (Fachbereich Biol., Univ. Regensburg, Regensburg, Ger.). Proc. Natl. Acad. Sci. U. S. A., 72(9), 3458-62 (English) 1975. CODEN: PNASA6.
AB ***Citrate*** ***lyase*** of Klebsiella aerogenes was dissocd. with urea. The 3 different subunits, .alpha.-chain (mol. wt. .simeq.54,000), .beta.-chain (mol. wt. .simeq.32,000), and .gamma.-chain (acyl carrier protein; mol. wt. .simeq.10,000), were isolated in pure and catalytically active state. Recombination of the 3 subunits produced ***citrate*** ***lyase*** that was indistinguishable from the untreated enzyme. The .alpha.-chain in the presence of acetyl-S-acyl carrier protein catalyzed the formation of the corresponding citryl thioester with liberation of acetate, and the .beta.-chain catalyzed the cleavage of citryl-S-acyl carrier protein with liberation of oxalacetate. A simple enzymic method for the prepn. of citryl-S-acyl carrier protein is described.

L32 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2002 ACS
1975:166684 Document No. 82:166684 Evaluation of the protein components of ***citrate*** ***lyase*** from Klebsiella aerogenes. ***Dimroth,*** *** Peter*** ; Eggerer, Hermann (Fachbereich. Biol., Univ. Regensburg, Regensburg, Ger.). Eur. J. Biochem., 53(1), 227-35 (English) 1975. CODEN: EJBCAI.
AB The specific activity of homogeneous and fully active ***citrate*** ***lyase*** from K. aerogenes was 55 units/mg protein, corresponding to .apprx.1.8 nmoles enzyme (mol. wt. .apprx.550,000). About 4-5 moles acylcarrier protein were present in the lyase. Evidence is presented which excludes the participation of the cysteine residue, present in the acyl carrier protein, as an acyl-carrying group. Thus, only the cysteamine residue of this subunit carries the acyl groups. The compn. of ***citrate*** ***lyase*** from 3 different proteins was confirmed. The mol. wts. of these subunits were .apprx. 10,000, 32,000, and 54,000; the latter mol. wt. was 56,000 by ultracentrifugation studies of the isolated, homogeneous protein. The molar ratio of the 3 subunits was .apprx. 1:1:0.8. This ratio indicates the presence of 6 copies of each of the 3 different subunits in the native enzyme complex, which probably consists of a hexamer.

L32 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2002 ACS
1975:134787 Document No. 82:134787 Composition of the ***citrate*** ***lyase*** carrier protein from Klebsiella aerogenes. ***Dimroth,*** *** Peter*** (Fachbereich Biol., Univ. Regensburg, Regensburg, Ger.). FEBS Lett., 51(1), 100-4 (English) 1975. CODEN: FEBLAL.
AB The amino acid compn. of the acyl carrier protein of ***citrate*** ***lyase*** from K. aerogenes was detd. and was very different from that of the Escherichia coli fatty acid synthetase, but the most marked difference was in the compn. and structure of the prosthetic groups. The prosthetic group of the acyl carrier protein from ***citrate*** ***lyase*** is a substituted isomeric dephospho CoA bound to a serine residue of the protein through a phosphodiester linkage of the 5'-phosphate group of adenylic acid. Another phosphodiester linkage binds 4'-phospho-pantetheine to the 2' or 3' position of ribose. The remaining OH-group of ribose is presumably substituted with a sugar residue.

L32 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2002 ACS
1975:120834 Document No. 82:120834 Mechanism of action of isocitrate lyase. ***Dimroth, Peter*** ; Mayer, Klaus; Eggerer, Hermann (Fachbereich Biol. Chem., Univ. Regensburg, Regensburg, Ger.). Eur. J. Biochem., 51(1), 267-73 (English) 1975. CODEN: EJBCAI.
AB The enzymes ***citrate*** ***lyase*** and isocitrate lyase catalyze similar reactions in the cleavage of citrate to acetate plus oxaloacetate and of isocitrate to succinate plus glyoxylate, resp. Nevertheless, the mechanism of action of each enzyme appears to be different. ***Citrate*** ***lyase*** is an acyl carrier

protein-contg. enzyme complex whereas isocitrate lyase is not. The active form of ***citrate*** ***lyase*** is an acetyl-S-enzyme but that of isocitrate lyase is not a corresponding succinyl-S-enzyme. In contrast to ***citrate*** ***lyase*** , the isocitrate enzyme is not inhibited by hydroxylamine nor does it acquire label if treated with appropriately labeled radioactive substrate. Isotopic exchange expts. performed in H2180 with isocitrate as a substrate produced no labeling in the product succinate. This was shown by mass-spectrometric anal. The conclusion drawn from these results is that no activation of succinate takes place on the enzyme through transient formation of succinic anhydride or of a covalently-linked succinyl-enzyme derived from this anhydride.

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1974:1123 Document No. 80:1123 Acyl-carrier protein of ***citrate*** ***lyase*** . ***Dimroth, Peter*** ; Dittmar, Wolfgang; Walther, Gundel; Eggerer, Hermann (Fachbereich Biol., Univ. Regensburg, Regensburg, Ger.). Eur. J. Biochem., 37(2), 305-15 (English) 1973. CODEN: EJBCAI.

AB Dissocn. of acetyl-14C ***citrate*** ***lyase*** gave a protein contg. phosphopantetheine. This protein component represents the acyl-carrier protein of ***citrate*** ***lyase*** (I). 14C-Acetylated acyl-carrier protein was purified. Inactivated, deacetylated I could also undergo the dissocn.-assocn. process. Recombination of these inactivated and dissocd. lyase species in the presence of purified acetyl acyl-carrier protein produced enzymically active I. An acyl-carrier protein present in crude exts. of Klebsiella aerogenes, but not the deacetylated, purified lyase acyl-carrier protein, was active in the malonyl-CoA-CO2 exchange reaction catalyzed by an Escherichia coli fatty acid synthetase prepn. lacking acyl-carrier protein indicating, that two different acyl-carrier proteins are present in K. aerogenes, one for fatty acid synthetase, the other for ***citrate*** ***lyase*** . It was assumed that 4 moles of acyl-carrier protein are present in 1 mole of the native complex which is addnl. composed of 16 protein subunits of identical or nearly identical size. The basic functional unit appears to be a pentamer contg. 1 acyl-carrier protein and 4 of these protein subunits. Hence, the native complex would consist of a tetramer of the functional basic pentamers. Lyase acyl-carrier protein contained 2 SH groups.